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--17. (new) The method according to claim 16, wherein said AMS gene is excisable, thereby to permit screening for said plants having integrated therein a transgene of interest.

--18. (new) The method according to claim 13, wherein said integrating step comprises transforming a plant with a plasmid vector containing said transgene of interest together with a promoter and a transcription terminator, said transgene being genetically linked with an AMS gene.--

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R E M A R K S

The present application has been amended in a manner that is believed to place it in condition for allowance at the time of the next Official Action.

Turning now to the issues raised in the Official Action, previous claims 1-12 are cancelled and a new set of claims 13-18 is introduced by the present amendment. New claims 13-15 correspond generally to the examined claims 1, 3 and 5, whereas new claims 16-18 recite further features of the present invention, in the context of the elected method.

In response to the criticisms as to the form of the abstract originally filed, set forth at Item 3 of the Official Action, the abstract is suitably amended herewith.

In response to the suggestion at Item 4 of the Official Action, appropriate section headings for the specification are also introduced by the present amendment.

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Responsive to the observation at Item 5 of the Official Action, a brief description of the drawings is introduced into the present specification by this amendment; and responsive to the requirement at Item 6 of the Official Action, the brief description of the drawings contains the appropriate sequence ID numbers for the sequences shown in the drawings, with the sequence ID numbers also being inserted at appropriate locations in the specification.

The concluding sentence of Item 6 calls for submission of the sequences in a computer readable form. That was already done, by the paper filed November 13, 2000 (copy of the postcard receipt attached). In the event that the computer readable form did not make its way to the Patent Office file, a duplicate copy is also attached to the present amendment.

Responsive to the observation at Item 7 of the Official Action, applicants respectfully submit that no essential material is incorporated by reference into the present application. Rather, the present disclosure, read in view of the knowledge provided by the relevant prior art, is sufficient unto itself to practice the claimed invention.

At Items 8 and 9 of the Official Action, claims 1, 3 and 5 previously in the case were rejected under 35 USC §101, on the basis that those claims were drafted in terms of a "use", which does not fall into one of the statutory categories set forth in §101. By the present amendment, that

informality of the original claims is corrected by converting the European-style "use" claims to U.S.-style "method" claims.

At Items 10 and 11 of the Official Action, previous claims 1, 3 and 5 were rejected under the first paragraph of 35 USC §112, as allegedly being based on a non-enabling disclosure. That rejection is respectfully traversed, for the following reasons.

The Official Action alleges that whereas the claims are drawn to prevention of dissemination of a transgene, the specification fails to provide guidance for effective prevention since this phenomenon is mostly unpredictable.

This assumption relies on a quotation of GRAY et al. (1998, Nature 392: 653-654) that discloses the possibility of transgene escape via seed spillage. However, the prevention of dissemination disclosed in the present application expressly relates to dissemination only by way of the pollen (page 2, lines 5-6; page 3, lines 15-17) New claims 13-8 are more explicit as to this point.

The main issue with transgenic plants growing in a field is the possible risk of cross-hybridization with wild species surrounding the field. Wild species could then be transformed with the transgene contained in the pollen of the transgenic plant. Therefore, accidental growing of a male sterile plant itself by seed spillage does not contribute to gene escape, since such a plant has no capacity to produce pollen.

Moreover, the specification refers to an article by Ellstrand dealing with the reduction of the risk of "escape" (page 1, line 30). This article only discloses the risk of transgene transfer through crop-weed hybridization. The risk of escape never refers to an accidental dissemination of the transgenic plant itself, such as seed spillage.

Therefore, the specification gives sufficient information for the person skilled in the art to prevent transgene dissemination through pollination.

In light of the above discussion, therefore, it is believed to be apparent that the non-enablement rejection as set forth at Item 11 of the Official Action has been overcome, and should not be repeated with respect to any of the new claims 13-18.

At Item 12 of the Official Action, previous claims 1, 3 and 5 were further rejected under the first paragraph of 35 USC §112, as allegedly failing to meet the deposit requirements of the Budapest Treaty as to certain plasmids contained in microorganisms, that were considered to be "essential" to the claimed invention. That rejection is also respectfully traversed, for the following reasons.

The plasmids described in the specification are in fact non-essential to the claimed invention: they are only disclosed as examples and are not exclusive of any other way to carry out the invention. Furthermore, a repeatable process to produce these plasmids is sufficiently disclosed in the specification. Indeed, restriction maps of the final con-

structured binary vectors are displayed in Figures 1 to 3, and for each intermediate plasmid developed, restriction enzymes to be used (for example KpnI/EcoRV and then KpnI/SmaI, KpnI/Sst I are used for plasmid pBIOC 500 construction, page 16) and detailed procedures have been described. See for example page 16:

"ligation was carried out at 14°C for 16 hours using 100 ng of the dephosphorylated plasmid and 50 ng of KpnI/EcoRV fragments in a 10 µl reaction medium in the presence of 1 µl of 10 x T4 DNA ligase buffer (Amersham) and 2.5 U of Tf DNA ligase enzyme (Amersham)."

Therefore, the information provided allows one skilled in the art to carry out the invention.

Consequently, it is believed that the above rejection under the first paragraph of 35 USC §112, should also be withdrawn and not repeated with respect to any of the new claims 13-18.

Concerning the indefiniteness rejection set forth at Item 14 of the Official Action, it is believed to be apparent that the newly-drafted claims 13-18 avoid each of the informalities noted by the Examiner with respect to original claims 1, 3 and 5. Note that, in new claim 15, the phrase "nuclear male sterility" is preserved, as sterility in this context is the noun itself. This usage is conventional and appropriate, as evidenced by the discussion in the specification, for example at page 2, line 21 through page 3, line 8.

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At Item 17 of the Official Action, previous claims 1, 3 and 5 were rejected as allegedly being obvious based on

PAUL et al. ("The isolation and characterisation of the tapetum-specific *Arabidopsis thaliana* A9 gene", Plant Molecular Biology, Vol. 19, 1992, pages 611-622) in view of ELLSTRAND et al. ("Hybridization As an Avenue of Escape for Engineered Genes", Bioscience, Vol. 40, No. 6, 1990, pages 438-442) and NYERS et al. ("Genetic Engineering of Reversible Sterility in Trees: Approaches, Problems and Progress", J. Cell Biochem., Vol. 15A, 1991, page 136"); and, at Item 18 of the Official Action, those claims were further rejected as allegedly being obvious based on WORRALL et al. ("Premature Dissolution of the Microsporocyte Callose Wall Causes Male Sterility in Transgenic Tobacco", The Plant Cell, Vol. 4, 1992, pages 759-771) in view of those same two secondary references. Those rejections are also respectfully traversed, for the following reasons.

The article by ELLSTRAND et al. is a general review about the risk of engineered genes escaping through hybridization. Among various strategies, the authors only briefly mention male sterility as an example of a genetic method of isolation. The publication does not disclose any further information about the way to put such a strategy into practice. Moreover, as revealed in conclusions, there was no evidence that pollination suppression would be effective for controlling transgene escape ("pollination studies may provide that strategies to reduce the escape of engineered genes via pollen are effective. However, these problems have yet to be explored", page 441, col. 3 §3).

The abstract from NYERS et al. mentions the use of genetic sterility as a way to prevent transgenes from entering natural gene pools. This abstract does not disclose the systems in which the transgene would be genetically linked to a sequence conferring male sterility. The general content of this short abstract relates to the agronomical advantages of reversible genetic sterility, in particular for forestry production ("sterile trees would have enhanced wood production", "multiplied for commercial plantings", "Production of seed...could be enhanced", "sanitary nuisance.") rather than to the environmental concern.

In any event, the sentence "genetic sterility **would** provide reliable and long lasting protection" (line 4) clearly indicates that the chances of success of such a strategy were not yet assessed.

Both PAUL et al. and WORRALL et al. describe the use of a gene system to achieve nuclear male sterility in tobacco, but none of them teaches the use of such a system to prevent transgene dissemination.

Remembering that the inventive step of an invention must not be assessed *a posteriori*, these articles, when considered as a whole, would not have encouraged one skilled in the art to prevent gene escape in the way of the present invention.

Even if the skilled artisan might have been motivated to attempt the use of nuclear male sterility to avoid transgene escape, there was no evidence that the use of such

a strategy would be successful at the time the present invention was made. Indeed, owing to the multiplicity of parameters involved in the progress, it was extremely difficult to anticipate the chance to prevent transgene escape from such a genetically engineered plant.

Therefore, the proposed combination of references applied in the outstanding Official Action will constitute at most a suggestion that it might be "obvious to try" a method such as that claimed. However, as held for example in the case of *In re Dow Chemical*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988):

The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art...both the suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure. (citations omitted).

Consequently, it is believed to be apparent that the proposed combinations of prior art applied at Items 17 and 18 of the Official Action, would not have rendered obvious the subject matter of previous claims 1, 3 and 5, and should not be applied in any rejection of any of the new claims 13-18.

In view of the present amendment and the foregoing remarks, therefore, it is believed that this application has been placed in condition for allowance with new claims 13-18.

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Allowance and passage to issue on that basis are accordingly respectfully requested.



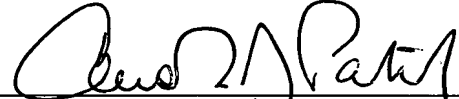
PEREZ et al. S.N. 09/380,086

Attached hereto is a marked-up version with changes made to the abstract and specification. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

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PEREZ et al. S.N. 09/380,086

ABSTRACT OF THE DISCLOSURE

The invention relates to novel uses of male sterility for improving the conditions for cultivating transgenic plants on behalf of man and the environment.

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

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Novel uses of male sterility in plants

10

DISCLOSURE

ABSTRACT OF THE TECHNICAL CONTENT OF THE INVENTION

15 The invention relates to novel uses of male sterility for improving the conditions for cultivating transgenic plants on behalf of man and the environment.

Fig.: none

was chosen was verified by enzymically digesting the plasmid DNA which had been introduced into it.

### EXAMPLE 13

Construction of a binary plasmid, pBIOC511, which carries male sterility, and selection on kanamycin between FRT specific recombination sites, utilizable in tobacco transgenesis.

10           The following sequences were deleted from the  
binary plasmid pGA492 (An, 1986): the constitutive  
promoter of the nos gene encoding nopaline synthase  
(Depicker et al., 1982), the sequence encoding the  
15   nptII gene, encoding neomycin phosphotransferase II  
(Berg and Berg, 1983), which sequence has been deleted  
of the region of the first 8 codons, including the  
methionine ATG start codon, and fused to the sequence  
of the first 14 codons of the sequence encoding the nos  
gene (Depicker et al., 1982), the sequence encoding the  
20   nos gene but lacking the region of the first 14 codons,  
and the nos terminator (Depicker et al., 1982). This  
deletion was carried out by total digestion with ClaI  
followed by partial digestion with SacII. The fragment  
is purified and then subjected to the action of the  
25   enzyme T4 DNA Polymerase (New England Biolabs) in  
accordance with the manufacturer's recommendations. The  
ligation of the plasmid and transformation of  
*Escherichia coli* DH5 $\alpha$  bacteria which had previously  
been rendered competent, are carried out in accordance  
30   with the customary methods (Sambrook et al., 1989). The  
resulting plasmid is termed pBIOC506.

35           The FRT specific integration site was amplified  
by PCR from the plasmid pOG45, which is marketed by  
Stratagene. The two oligodeoxynucleotides employed as  
primers for the PCR are:

5' CCC CTG CAG TTT TCA AAA GCG CTC TGA AGT TC 3' and

5' CCA AAG CTT GAA TTC GCC AGG GGG ATC TTG AAG TTC 3'

The fragments which corresponded to the FRT site, and which were derived from the PCR amplifica-

(SEQ ID NO: 1)  
1  
(SEQ ID NO: 2)  
1

tion, were digested with PstI, subjected to the action of the enzyme T4 DNA Polymerase (New England Biolabs) in accordance with the manufacturer's recommendations, and then digested with EcoRI. They were purified on a 2% agarose gel, after which they were electroeluted, precipitated with alcohol, dried and taken up in water. They were then ligated between the SacI site, which had previously been subjected to the action of the enzyme T4 DNA Polymerase, and the EcoRI site of plasmid pUC18, which is marketed by Pharmacia. The resulting plasmid is termed pBIOC507.

A second FRT site was introduced into plasmid pBIOC507. This second FRT site results from carrying out a PCR amplification using the two oligodeoxynucleotides:

5' CCC CTG CAG TTT TCA AAA GCG CTC TGA AGT TC 3' and  
5' AAA GGT ACC GCC AGG GGG ATC TTG AAG TTC 3'.

(SEQ ID NO: 1)  
1  
(SEQ ID NO: 3)

The amplified fragments were digested with PstI and KpnI, subjected to the action of the enzyme T4 DNA Polymerase, and ligated in the SphI site, which had previously been treated with the enzyme T4 DNA Polymerase, of plasmid pBIOC507. The resulting plasmid is termed pBIOC508 and contains the two FRT sites oriented in the same direction.

The HindIII/EcoRI fragment carrying the two FRT sites was isolated from plasmid pBIOC508. This fragment was inserted at the HindIII and EcoRI sites of plasmid pBIOC506. The resulting plasmid is termed pBIOC509.

The SphI fragment carrying the chimeric gene corresponding to the "A9-barnase-T35S promoter" contained in pWP173 (Paul et al., 1992) was used for conferring male sterility. This SphI fragment was treated with the enzyme T4 DNA Polymerase and ligated into the PstI site, which had been treated with the enzyme T4 DNA Polymerase, of plasmid pBIOC509. The resulting plasmid is termed pBIOC510.

Finally, in order to permit selection on kanamycin (Fromm et al., 1986), the EcoRI fragment, which had been treated with the enzyme T4 DNA